Supercritical Fluid Chromatography

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The role of supercritical fluid chromatography (SFC) in the analysis of foods and agriculturally derived products has been somewhat moderated by uncertainties in the availability of required instrumentation for the past 15 years. In addition, SFC competes for the same analytical opportunities as gas (GC) and high performance liquid chromatography (HPLC) and hence is often ignored or relegated to a minor role by food analysts. Despite these difficulties, SFC has been applied to a variety of applications for the detection and quantification of analytes, that are at least soluble to even a minor extent in supercritical carbon dioxide ($SC\text{-}CO_2$) – by far the most popular mobile phase utilized in the technique.

The application of SFC to food matrices came naturally due in part to the early application of $SC\text{-}CO_2$ extraction in the food industry, i.e. for the

extraction of coffee, hops and similar food items used routinely by the consuming public. SFC is particularly applicable to the analysis of lipid-containing materials, due to relative high solubilities exhibited by these solutes (analytes) in SC-CO_2. Analysis and detection of ultra-trace components in foodstuffs, e.g. pesticides or drugs, has not been generally successful because of the problems in routinely interfacing and using sensitive detectors, such as the electron-capture detector (ECD) with SFC, due to the change in mobile-phase characteristics with respect to time during the analysis. However, the ability routinely to use the flame ionization detector (FID) with SFC has provided the analyst with a useful technique to detect an array of components, or a specific moiety, in complex food matrices.

With respect to the chromatographic technique utilized, it is capillary SFC which has been cited more often then packed-column SFC in the analysis of foods. This is somewhat unfortunate since the packed-column mode also offers interesting possibilities, particularly when interfaced with a ultraviolet (UV) detector or evaporative light-scattering detector (ELSD). The recent use of this technique for the analysis of chiral compounds may also create some opportunities in food analysis, where the knowledge of the chirality of certain compounds (e.g. flavour esters) is of importance. In general, the coupling of analytical supercritical fluid extraction (SFE) with SFC has not been adopted to any considerable extent by food analysts, due to the lack of an interface that permits routine coupling and use of the SFE/SFC mode. However, preparative and even production scale SFC has been utilized for specialized applications in the food production industries, and probably will see increased application due to the current interest in producing high value nutraceutical components, in a natural and environmentally benign manner.

Since SFC is perceived as a niche technique in the food industry, it is important to recognize when and where it can be used to advantage relative to what can be achieved using GC and HPLC. Some of these opportunities are as follows:

1. Reduction of the use of organic solvents relative to HPLC;
2. Direct analysis of samples avoiding sample preparation steps;
3. Deformulation of commercial food products;
4. Detection of product adulteration or deterioration;
5. Support of food engineering extraction/reaction process development.

With respect to nonpolar solutes, pressure-or density-programmed SFC provides the capability to analyse compounds having molecular weights approaching 1200 amu in one chromatographic analysis. Separation of these compounds is a function of their

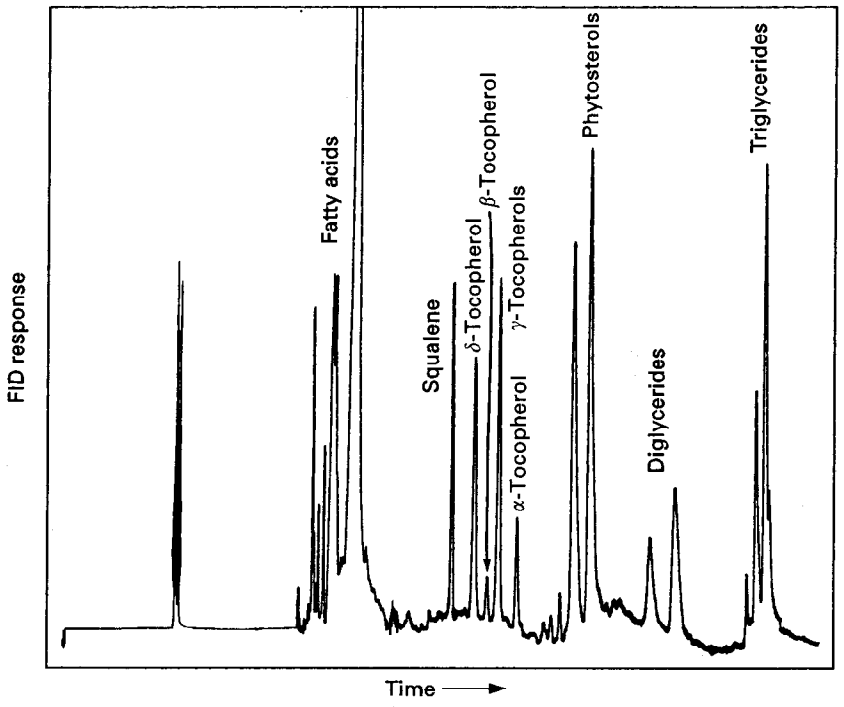

Figure 1 Supercritical fluid chromatography analysis of deodorizer distillate with an SB-octyl-50 column using flame ionization detection (FID).

solubility in the mobile phase, their respective vapour pressures and miscibility of the solute in the fluid phase. For example, in **Figure 1**, a number of components have been separated using a capillary SFC method that traditionally would have required the use of both GC and HPLC, and derivatization of some of the analytes. Utilizing SFC allows the analyst to avoid the above approaches, and to analyse directly the sample, obtaining a snapshot of the entire molecular composition. These characteristic elution patterns produced by SFC can be used to identify the presence or absence of a particular molecular constituent in a food sample, thereby providing valuable information for the food product formulator, to match or alter in developing new and competitive products.

Increasing concerns about minimizing or eliminating the use of hazardous organic solvents in the laboratory also bodes well for the application of SFC. Incorporating SFC for the separation and detection of food-related solutes, eliminates not only most of the traditional solvent needs associated with HPLC, but any solvents utilized in the extraction or sample work-up steps prior to analysis. In this regard, SFC is an excellent tool for monitoring the end-result of an extraction or reaction of a food component using supercritical fluid media. Also, by using SFC, food-related analytes that are thermally labile or susceptible to degradation via oxidation are not exposed to the harsh conditions that often accompany their analysis by GC or HPLC. This advantage can be attributed to the protective action of CO_2 which excludes oxygen, and the low temperatures used when separating components via SFC.

Selecting and Optimizing Separation Conditions for Food Components

For the SFC analysis of food-related samples, the analyst will undoubtly want to start with a general fluid-programming sequence to interrogate the sample matrix as to its components. These programmes are executed for an extended time to ensure optimum resolution and detection of the unknown or target analyte(s). Run times of 90 min in length are not unusual in this initial stage of method development. After the target analytes have been identified by retention time-matching with standards, or via an independent method such as mass spectrometry (MS), the original programme can be modified to reduce the analysis time or improve the resolution within the chromatogram. However, changes in the mobile-phase programme will usually be done to hasten the elution of early or late eluting components that are of no importance in the analysis. In the analysis of food components, both changes in the rate of increase of the fluid pressure or density with respect to time, or in some cases changeover to an exponentially-based fluid programme, will suffice to optimize the SFC run.

Because of the molecular complexity exhibited by many food ingredients and compositions, it is not unusual to have a temperature gradient with respect to time superimposed on the mobile-phase pressure programme during the SFC run. For example, separation of the like-carbon number triglycerides in soybean oil is not possible by pressure or density programming alone, but by superimposing a temperature gradient during the analysis, these oil components can be well separated. SFC analysis under isobaric conditions is limited in application when analysing foods; however, it should not be overlooked since it can often yield the most precise and accurate results.

The FID is the most commonly used detector for SFC. FID sensitivity to food components is lower than that obtained with GC since expansion of the mobile phase dilutes the detector signal substantially. However, the FID signal can be amplified to permit analysis down to the p.p.m. level, provided any shift in the baseline can be compensated for. Analytes with chromaphoric properties are amenable to UV detection in conjunction with SFC. The absorption maxima of components shift as a function of fluid density or pressure, but most detector units constructed for operation at these elevated pressures allows for stop flow or *in situ*, on-the-fly scanning of peaks to determine absorbance maxima shifts. For example, bathochromic shifts of 15–20 nm have been recorded for carotenoids over a 250 atm pressure interval in SC-CO_2. The mating of the ELSD detector with SFC has been reported by several investigators; however, day-to-day stability is inferior to that experienced with HPLC-ELSD couplings when applied to food analysis. Hetero-element-specific detectors, such as ECD or flame photometric detector, have mostly been utilized in research studies using SFC and have not seen serious adoption for routine analysis. Again, detector sensitivity and stability under SFC conditions limit their sensitivity at best to parts per million range. The use of SFC with mass spectroscopy has remained mainly an academic art, and commercial instrumentation development has been limited to date.

Most of the promising applications of capillary SFC have utilized nonpolar bonded phases such as methyl, octyl, phenyl and biphenyl silicas. The weak elutropic strength of neat SC-CO_2 has favoured the use of short chain length; monomeric silane-modified columns have C_1, C_4, C_{18}, phenyl, amino and diol

phases for packed-column SFC. The choice of these phases is not so much related to their selective interaction with food-related solutes, but to their surface-modifying properties which reduce peak tailing and solute interaction with the silica matrix. Resin columns have also been utilized, but they are susceptible to voiding unless specifically packed for use under supercritical fluid conditions.

Types of Food Components Analysed by SFC

A myriad of food-related components and matrices have been analysed by SFC, as indicated by the partial listing in **Table 1**. These include naturally occurring ingredients such as fats/oils, spices, etc.; minor unwanted constituents like pesticides, antibiotic drugs and mycotoxins; and specific food components, including nutraceuticals and flavouring aids. Inspection of Table 1 indicates a preponderance of applications in the lipid analysis area. Indeed, SFC is tailor-made for lipid analysis, although somewhat lacking in the high resolution capabilities demonstrated by high temperature GC. The retention pattern for lipid solutes in SFC, as shown in Figure 1, follows a distinct pattern governed approximately by the solute's molecular weight/volatility characteristics. Elution of the following classes of lipids is in the order: fatty acid methyl esters, free fatty acids, hydrocarbons, vitamins, sterols, wax esters, mono- followed by diglycerides, and then triglycerides/steryl esters. Although there is some overlap between individual classes of the above solutes, due to the overlapping molecular weights ranges (e.g. triglycerides and steryl esters), this separation pattern has proven very useful in tracking conversion of lipid species undergoing reaction as well as in the quality control of food raw products and ingredients.

Triglyceride-based oils/fats are also readily amenable to analysis by SFC. Separation of the individual components is once again governed by molecular weight considerations, thereby allowing SFC to facilitate the separation of the major triglyceride species, i.e. T_{50}, T_{52}, T_{54}, etc. For some oils, such as coconut oil, will-resolved chromatograms result, while for other oils, e.g. soybean oil, there is overlap between the saturated and unsaturated triglyceride species, making superimposition of temperature gradient along with the pressure gradient programme for the mobile phase necessary to achieve adequate resolution. However, even without ideal resolution, the rapid analysis afforded by SFC can be used to considerable advantage for quality control, where speed, rather than optimal separation, is often desired.

Detection of minor components in foods is limited by the detector stability problems noted previously; however, those components which can be detected by using FID, UV or ELSD are often analysed more rapidly by SFC, due to the time savings afforded by avoiding elaborate preparation of the sample prior to analysis. SFC analysis provides a more detailed profile of the entire sample in addition to detecting the target analyte. This allows a more accurate assessment of the total contribution of the minor constituent to the entire ingredient profile, e.g. the presence of sterol esters in sawtooth palmetto berry extracts, where fatty acids and triglycerides are the major constituents.

Other food sample types that are readily analysed by SFC are the fat-soluble vitamins, essential and flavour oil ingredients, spice materials, hop compo-

Table 1 Food components separated and analysed by SFC

Carbohydrates	Derivatized corn syrups, mannose glycans
Chiral compounds	Monoterpenes, pyrazines, clenbuterol
Drugs/antibiotics	Caffeine, erythromycin, polycyclic ether antibiotics, sulfonamides, assorted steroids
Hydrocarbons	Sesquiterpenes, squalene, waxes and wax esters
Lipids	Fatty acids, fatty acid esters, monoglycerides, diglycerides, triglycerides, sterol esters, sterols (cholesterol), fat-soluble vitamins, tocopherols, phospholipids (lecithin), lipid hydroperoxides, glycolipids
Nutraceuticals	Valeriana, gingolides, sawtooth palmetto berry
Oils/fats	Celery oil, coconut, fish, soybean, wheat germ, palm oil, rice oil, milk/cheese triglycerides
Packaging/film components	Polypropylene oligomers, polyvinyl chloride, phenolic antioxidants, low molecular weight polystyrene
Pesticides	Halogenated, organophosphorus, carbamate, pyrethrins, acidic phenoxy herbicides, sulfonyl ureas
Pigments	Carotenoids, xanthophylls
Speciality ingredients	Hops components
Spices/flavours	Capsicum, cardoman, coumarin, curry, garlic components, majoran, rosemary, vanillin
Terpenes/essential and fruit oils	Grapefruit oil, limonenes, mint, lemon
Other toxicants	Mycotoxins, nitrosamines, polycyclic aromatic hydrocarbons

nents and nutraceutical formulations. Some SFC-based separations require the use of a co-solvent (usually 5–20 vol%) in addition to the SC-CO_2 for the mobile phase. For example, phospolipids are only sparingly soluble in neat SC-CO_2, but these polar lipid compounds can be chromatographed successfully on packed silica columns by incorporating ethanol and/or water as a modifier into the mobile phase. Likewise, carbohydrate moieties, which exhibit limited or no solubility in SC-CO_2 or SC-CO_2/co-solvent mobile phases, can be derivatized to allow their analysis by SFC.

Selected Applications of SFC in Food Analysis

In this section, several brief examples will be given to illustrate the utility and potential of SFC in food analysis. Figure 2 illustrates the SFC separation and detection of α-tocopherol and cholesterol in a fish oil capsule. This was achieved on a capillary SB-methyl column at 120°C using the density programme noted on the horizontal axis. Although this analysis took over 90 min to perform, it illustrates some of the benefits that can be achieved using SFC. For example, the chromatogram in Figure 2 was achieved with no sample preparation other than to dilute the oil in a small quantity of solvent and to inject it into the chromatograph. In addition, no derivatization of the sample was required and adequate resolution between the α-tocopherol and cholesterol was achieved using the lengthy density programme. However, it is perhaps more important that, by adjusting the elution conditions, the background components (fish oil triglycerides) that were of no interest in this analysis can be programmed off the column without resorting to a pre-fractionation of the sample prior to SFC analysis or derivatization of the sample matrix.

Not all applications of SFC require the above conditions for high resolution separations. For example, packed-column SFC (5 μm, C_8 Deltabond) has been used to clean up samples prior to other types of chromatographic analysis (GC). In this case, organochlorine and organophosphorus pesticides were extracted by SFE with SC-CO_2 from a meat sample, and the pesticides separated from the co-extracted fat moieties using the packed SFC column. Hence, by 'heart-cutting' the appropriate elution fraction, a lipid-free, pesticide-containing fraction was provided for GC residue analysis.

SFC is an excellent technique to monitor reaction chemistry between lipid species, since it avoids the need to employ more than one analytical technique or sample derivatization. Further, it permits the successful chromatography of all of the relevant reactants and products in one chromatographic analysis. Examples where SFC has been applied are in the esterification or transesterification of lipids, glycerolysis reactions and randomization of fats/oils. Table 2 shows the analysis of the glyceride content of

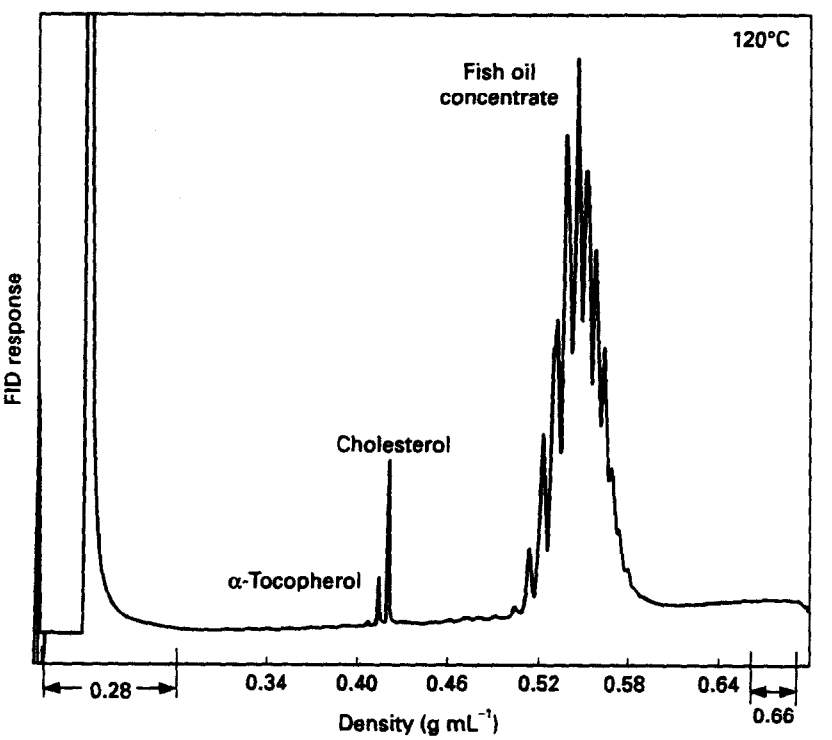

Figure 2 Determination of cholesterol and α-tocopherol in a fish oil capsule by capillary SFC.

Table 2 Analysis of glycerides in a randomized fat sample

Methods of analysis	*MG*	*DG*	*TG*	*Time of analysis*
SFC-FID	0.2	9.6	90.1	25 min
GC-FID	0.1	6.9	92.9	30 min
HPLC-FID		13.5	86.5	1 h
HPLC-ELSD		8.0	92.0	30 min
LC-silica column	1.0	7.7	93.1	2 h
TLC	2.0	11.0	87.0	30 min

MG, Monoglyceride; DG, diglyceride; TG, triglyceride.

a randomized fat sample using six different analysis methods. The results given in Table 2 suggest that SFC-FID analysis yields comparable data for an equivalent analysis time to that obtained using the GC-FID and HPLC-ELSD methods. However, the SFC method does not require the time and effort for sample preparation associated with the alternative techniques and, in addition, saves on the cost of solvents and chemical reagents. A further illustration of the cost- and time-saving advantages of SFC is noted by its ability to monitor free and methylated fatty acids, thereby providing a reasonably quick and accurate assay for these compounds in foodstuffs to support nutritional analysis claims and the detection of frying oil deterioration as a function on time.

Preparative or production scale SFC is now being used as a separation technique in the food industry. Fractionation and isolation of higher value food components, such as tocopherols and phospholipids, or the ω-fatty acids/esters from fish oils, have been cited in the literature. Recently, a production plant for the separation of fish oil ethyl esters has been constructed in Spain to produce $\geq 95\%$ pure polyunsaturated fatty acids for the nutraceutical market. The basic separation design of this production scale plant is based on chromatographic fractionations initially developed using analytical scale packed SFC columns.

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